

CLAIMS

What is claimed is:

- 5 1. A hybrid nucleotide sequence of no more than 1528 base pairs including a sequence defining a structural gene expressing a conjoined single strand of a multimeric TNFSF-SPD fusion protein, said structural gene having a nucleotide base sequence selected from members of the group consisting of SEQ ID NO 1, SEQ ID NO 3 and SEQ ID NO 5.
- 10 2. The DNA segment according to claim 1, wherein the structural gene comprises:
 - a segment expressing a single hybrid amino acid chain of TNFSF-SPD, said segment having
 - a first SPD nucleotide base sequence of SEQ ID NO 1, from base 32 to base 799, and
 - a second sequence, expressing a portion of TNFSF stalk, selected from members of the group consisting of SEQ ID NO 1, from base 800 to base 1444, SEQ ID NO 3, from base 800 to base 1528, and SEQ ID NO 5, from base 800 to base 1441.
- 20 3. A recombinant DNA molecule comprising a vector operatively linked to an exogenous DNA segment defining a structural gene expressing a single amino acid chain of TNFSF-SPD, said structural gene having
 - a nucleotide base sequence selected from members of the group consisting of SEQ ID NO 1, SEQ ID NO 3 and SEQ ID NO 5, any functional equivalents and modifications thereof, and

an appropriate promoter for driving the expression of said structural gene in a compatible host organism.

4. The recombinant DNA molecule as described in claim 3 wherein said host organism
5 is *E. coli*.

5. The recombinant DNA molecule as described in claim 3, wherein said host organism is a yeast.

10 6. The recombinant DNA molecule as described in claim 3, wherein said host organism is a higher plant or animal.

Exhibit B2
7. A multimeric TNFSF-SPD fusion protein comprising:
a plurality of polypeptide trimers,

- 15 i) a first trimer consisting of peptide strands of members of the
TNF superfamily (TNFSF) of ligands, and
ii) a second trimer strand from a collectin molecule

20 each first trimer conjoined to a second polypeptide trimer strand from
a collectin molecule, wherein said ligand strand is substituted for native
carbohydrate recognition domains (CRD) of the collectin molecules,

25 said conjoined collectin strands covalently bound in parallel to each
other, therein forming a multimeric fusion protein comprising a plurality of
trimeric hybrid polypeptide strands radiating from a covalently bound center
hub of the molecule, the free end of each trimeric radiating strand having a
TNFSF moiety attached.

Sub-a > 8. The multimeric fusion protein according to claim 6, wherein the TNFSF moiety is one selected from the group consisting of ligands LTA, TNF, LTB, and TNFSF4 to TNFSF 18 as in Table II, their functional equivalents, and modifications thereof.

5 9. A method for preparing a CD40-SPD multimeric fusion polypeptide, comprising the steps of:

initiating a culture, in a nutrient medium, of prokaryotic or eukaryotic host cells transformed with a recombinant DNA molecule including an expression vector, appropriate for said cells, operatively linked to an exogenous DNA segment defining a structural gene 10 for CD40-SPD ligand, said structural gene having a nucleotide base sequence of SEQ ID NO 1 from about base 32 to about base 1444; and

maintaining said culture for a time period sufficient for said cells to express said multimeric molecule.

15 10. A method of producing a secreted, large, biologically active, multimeric tumor necrosis factor superfamily ligand fusion protein chimera that is highly immunogenic and not readily diffusible, comprising:

introducing into a host cell a first chimeric DNA construct including
a transcriptional promoter operatively linked to a first secretory signal
sequence, followed downstream by, and in proper reading frame with
20 a first DNA sequence encoding a polypeptide chain of a first
TNFSF ligand requiring multimerization for biological activity,
joined to

a second DNA sequence encoding a collectin polypeptide at
the site where the collectin's CRD was purposefully removed,

25 introducing into said host cell a second DNA construct including

a transcriptional promoter operably linked to a second secretory signal sequence followed downstream by, and in proper reading frame with,

5 a third DNA sequence encoding a second polypeptide chain of a second TNFSF ligand, joined to

a fourth DNA sequence encoding a collectin polypeptide, wherein the collectin's CRD was purposefully removed;

10 growing said host cell in an appropriate growth medium under physiological conditions to allow the secretion of a large multimerized polypeptide fusion protein, wherein said first polypeptide chain of a TNFSF-SPD protein is bound by parallel bonding of the respective collectin domain trimer to

15 said second polypeptide chain of a different TNFSF-SPD polypeptide trimer, and wherein said multimerized polypeptide fusion protein exhibits biological activity characteristic of both membrane-attached TNFSFs; and

isolating said biologically active, multimerized TNFSF-SPD polypeptide fusion from said host cell.

11. The method according to claim 10, wherein the chimeric reactant compounds are humanized to guard against destruction by a potential human recipient's immune system.

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12. A method of preparing a multimeric TNFSF-SPD ligand fusion protein, comprising:
preparing a first DNA segment coding for a strand of an exposed extracellular portion of TNFSF;
preparing a second DNA segment coding for a collectin polypeptide strand, wherein said collectin's CRD domain of the strand has been removed;
25 conjoining said first and second DNAs in proper reading frame, therein creating

a TNFSF-collectin DNA construct;
inserting said construct into an expression vector system;
introducing said vector system into an appropriate cell in culture under suitable
conditions;
5 harvesting and purifying spent medium from said culture; and
assaying for presence of multimeric TNFSF-collectin fusion protein.

13. A method for stimulating the immune response in potentially immunocompetent
cells using multimeric TNFSF fusion proteins, comprising:
10 contacting said cells with said multimeric TNFSF fusion proteins, wherein said cells
are induced to proliferate.
14. The method according to claim 15, wherein the cells are resting B cells.
- 15 15. A method for increasing antigenicity of cells, comprising:
contacting said cells with said multimeric TNFSF fusion proteins, wherein said cells are
tumor cells or HIV positive cells.